UNCLASSIFIED

AD NUMBER

ADB216726

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to DoD only; Specific Authority; Oct 96. Other requests shall be referred to U.S. Army Medical Research and Materiel Command, Attn: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

AUTHORITY

USAMRMC ltr, 4 Dec 2002

THIS PAGE IS UNCLASSIFIED

AD ______________

CONTRACT NUMBER DAMD17-96-C-6046

TITLE: Development of an Insect Repellent Based on Human Skin Emanations

PRINCIPAL INVESTIGATOR: William G. Reifenrath, Ph.D.

CONTRACTING ORGANIZATION: Reifenrath Consulting and Research
Richmond, California 94806

REPORT DATE: October 1996

TYPE OF REPORT: Final-Phase I

19961202 085

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

DISTRIBUTION STATEMENT: Distribution authorized to DOD Components only (specific authority). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
October 1996

3. REPORT TYPE AND DATES COVERED
Final-Phase I (15 Mar 96 - 14 Sep 96)

4. TITLE AND SUBTITLE
Development of an Insect Repellent Based on Human Skin Emanations

5. FUNDING NUMBERS
DAMD17-96-C-6046

6. AUTHOR(S)
William G. Reifenrath, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Reifenrath Consulting and Research
Richmond, California 94806

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-25012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to DOD components only (Specific Authority). Other requests for this document shall be referred to Commander, U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RMI-S, Fort Detrick, Frederick, MD 21702-5012

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

During Phase I, we have identified human subjects from a group of 30 males and females whose forearms were consistently least attractive or most attractive to Aedes aegypti mosquitoes contained in an olfactometer ($F = 5.92$, $P = 0.0000$). All of the 4 least attractive subjects were female and all of the 5 most attractive subjects were male. Females in general were significantly less attractive to the mosquitoes than the males (ANOVA, $F = 49.33$, $P = 0.0000$).
Several pilot studies were conducted in Phase I. First, we demonstrated that Aedes aegypti attraction to the forearm of a male subject could be essentially extinguished by thorough washing of the forearm. Second, volatile emanations from a subject's forearm could be captured on solid adsorbent; thermal desorption into the olfactometer demonstrated their attractancy. Third, volatile emanations from a subject's forearm could be captured in a canister and analyzed by gas chromatography mass spectrometry. Fourth, we developed a model for human attractancy to mosquitoes using excised pig skin. This model was either very attractive or essentially unattractive to mosquitoes, depending on the time of measurement.
In summary, we have 1) developed both in vitro (pig skin) and in vivo (human subjects) test systems whose attractancy to mosquitoes varies in a predictable way and 2) demonstrated appropriate methodology for collection and analysis of skin emanations.

14. SUBJECT TERMS
Mosquito, Attractant, Repellent, Skin, Emanations

15. NUMBER OF PAGES
37

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

( ) Where copyrighted materials is quoted, permission has been obtained to use such material.

( ) Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

(X) Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

( ) In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

(X) For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 32 CFR 219 and 45 CFR 46.

( ) In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

William G. Reifenrath
Principal Investigator's Signature

October 1, 1996
Date

Table of Contents

INTRODUCTION

At the present time, the only chemical used commercially as a topical insect repellent is N,N-diethyl-m-toluamide (DEET). The current US Army insect repellent (EDTIAR) contains DEET as its active ingredient. DEET has a limited spectrum of activity and a noticeable odor that is unpleasant to many. DEET is a powerful plasticizer and will dissolve or mar many plastics and painted surfaces. DEET will plasticize the polymers that are typically used in topical formulations to extend its duration, leading to formulations with low user acceptability.

There is ample evidence that human skin emanates both attractant and repellent compounds for mosquitoes. No single compound is likely responsible for mosquito attraction; the same can be said for mosquito repulsion. The interaction of these compounds is probably of importance in the overall response of the mosquito. Studies of human skin emanations associated with mosquito attraction/repulsion have been reviewed (Appendix A).

Skin emanations have been poorly characterized[1]. While a few studies have chemically characterized skin surface extracts, it is important to characterize what the skin is actually emanating; that is, what chemicals the mosquito actually senses during its host seeking behavior, rather than residues on the skin. Some studies have utilized a solvent evaporation step in the process of characterizing skin residues[2]; important volatile components could have been lost in the process. Finally, gas chromatographic-mass spectrometric techniques have been greatly advanced since the earlier studies were conducted, both in terms of sensitivity and software for analysis of mixtures.

To begin this project, we identified subjects who were consistently very attractive or unattractive to mosquitoes. Additionally, we developed an in vitro model to study host attraction and conducted pilot studies to evaluate methods for emanation collection and analysis. Phase II investigations with modern analytical techniques will unravel the chemical identity of both host attractants and repellents. Armed with this knowledge, a new repellent can be designed which amplifies natural host repellents and masks host attractants.

MATERIALS AND METHODS

Olfactometer: A Fiensod and Spielman olfactometer, as modified by Bowen and Davis, measured the host-oriented flight response of female mosquitoes to volatile host emanations[3]. The olfactometer (approximately 38 cm high) consisted of an upper and lower screened chamber with a closure between the chambers (Figure 1). A fan placed above the upper chamber drew air through the apparatus at approximately 0.2 m/s. A temperature and humidity controlled chamber (5' wide by 6'long by 8'high) was constructed to house the test subject and the olfactometer.

Rearing of mosquitoes: A second environmental chamber, maintained at 27°C and 80% humidity, was dedicated to the rearing of Aedes aegypti mosquitoes. Routine shipments of eggs (American Biological Supply, Gainesville, FL) were used to maintain a continuous supply of adult 5-10 day old mosquitoes.

Assays for attraction of mosquitoes to human subjects: A group of 30 volunteers, consisting of 14 females and 16 males and ranging in age from 24 to 68 years, was selected from the surrounding civilian population. Individuals were tested for their ability to attract Aedes aegypti mosquitoes contained in the olfactometer. Tests were conducted at a temperature of 27°C and 50% relative humidity. For each trial 15 avid adult female Aedes aegypti mosquitoes (5-10 days post-emergence) were placed in the upper chamber. A trial began when the closure between the upper and lower chamber was opened in the absence of a human host. The number of mosquitoes entering the lower chamber within a 3 minute period was recorded. The volunteer then placed his or her arm beneath the lower chamber and the number of mosquitoes flying from the upper chamber to the lower chamber was recorded for the time intervals 0-1, 1-3, 3-5 and 5-7 minutes. This trial was repeated twice during a test session to obtain three replicates. Two additional test sessions, at time intervals of at least 1 week, were conducted to obtain at least 8-9 replicates for each of 24 subjects. Of the remaining 6 subjects, 3 were tested on two separate occasions for a total of 6 replicates per subject; 3 were tested on one occasion for a total of 3 replicates per subject. A total of 254 tests were conducted.

Calculation of olfactometer scores: Olfactometer scores were calculated for each trial by dividing the number of mosquitoes entering the lower chamber of the olfactometer during the 0-1, 1-3, 3-5 and 5-7 minute intervals by the number of mosquitoes remaining in the upper chamber of the olfactometer at the end of the 3 minute control period. The fractions so obtained was plotted versus time. An equation was fitted to the data and the area under the curve (olfactometer score) was calculated. An area of 0 (0 mosquitoes entering the lower chamber x 7 minutes) would indicate the subject was completely unattractive to mosquitoes. An area of 7 would indicate maximum attraction.

Collection and desorption of skin emanations: A glass "arm tube", 4 inches in diameter and 20 inches in length, was wrapped with heating tape to maintain a temperature of approximately 37°C (rheostat setting of 20 volts). One end of the tube allowed the insertion of a forearm, while the terminal 4 inches of the other end tapered to a tube with an inside diameter of 0.25 inch. The tube was attached to one end of a vapor trap, constructed from a glass tube, 1/2 inch in inside diameter and 6 inches in length. The vapor trap was packed with approximately 5 grams of adsorbent powder (Tenax GC, 60/80 mesh, Alltech Associates, Deerfield, IL), which was held in place with silanized glass wool (Supelco, Bellefonte, PA). The other end of the vapor trap was attached to an air flow gauge, which was connected to a small rotary vane vacuum pump. Air flow through the apparatus was 1 liter per minute. Prior to use, the arm tube was rinsed with ethanol, drained and allowed to dry for two hours with the help of the heating tape. Prior to emanation collection, the vapor trap was connected to a supply of nitrogen gas (99.9999% minimum purity, Matheson Gas Products, Newark, CA) at a flow rate of 40 ml/min. The vapor trap was positioned so that its outlet was approximately 2 inches below the lower chamber of the olfactometer. The vapor trap was wrapped in heating tape (140 watt) and was thermally desorbed into the olfactometer by connection of the heating tape to a rheostat set at 25 volts. The desorption period was 7 minutes following a 3 minute control period at ambient temperature. After cooling, the vapor trap was reconnected to the arm tube and the forearm of subject 02 was positioned inside the tube. Ambient air was allowed to enter the arm tube from a side port near the arm entrance opening of the arm tube. Emanations were collected for a period of 20 minutes. The vapor trap was disconnected from the arm tube, reconnected to the nitrogen gas, and thermally desorbed into the olfactometer.

<u>Gas chromatography/mass spectrometry of skin emanations</u>: The arm tube was prepared as described in the preceding section "Collection and desorption of skin emanations". The tapered end of the arm tube was fitted with a 1/4 inch Swagelok nut for connection to a Summa canister. The side port near the open end of the arm tube

was connected to the source of nitrogen and the arm tube was heated and purged with nitrogen gas for 1.5 hours. A Summa canister was prepared by repeated filling with helium and evacuating with a pump. The Summa canister was connected to the arm tube and the forearm of subject 02 was positioned inside the tube so that the upper forearm sealed the open end of the arm tube. Nitrogen gas flowed into the arm tube at 500 ml/min and was captured by opening the valve on the evacuated Summa canister. The collection period was 5 minutes. After sample collection, the canister was transported to the Varian Associates application laboratory in Walnut Creek, CA. The canister was connected to a Varian Saturn 3 GC/MS and a 250 ml portion of the sample was drawn into the gas chromatograph through a mass flow valve. The sample was preconcentrated on liquid nitrogen-cooled glass beads at the head of the capillary column. Rapid heating (approximately 40°C/sec) of the cryogenic trap provided fast injection of the condensed emanations into the capillary column. Only 1/10 of the condensed sample entered the capillary column; the remainder was vented to the atmosphere. Components were separated on a 60 m, 0.32 mm inside diameter DB-1 capillary column with a 1 um film thickness. The mass spectrometer provided full scan library-searchable classical EI spectra of compounds. A protocol similar to EPA TO-14[4] for monitoring air quality was used.

<u>Attractancy of excised pig skin</u>: Split thickness pig skin, prepared from full thickness skin with a dermatome, was used at a

thickness of 0.5-1.0 mm. It was mounted on a diffusion cell (Figure 2) so that an approximately 5.0 cm diameter circle of skin was exposed to the atmosphere. The visceral side of the skin was bathed in RPMI tissue culture media. Water at 37°C circulated through the jacket of the diffusion cell to maintain the skin surface at approximately 32°C[5]. Fifteen female Aedes aegypti (5-10 days old and sugar deprived for 24 hours) were placed in the upper chamber of the olfactometer. After a 3 minute control period to determine the number of mosquitoes entering the lower chamber due to random flight, the excised pig skin/diffusion cell assembly was positioned approximately 1 inch below the lower chamber of the olfactometer. The number of mosquitoes entering the lower chamber during the time intervals of 0-1, 1-3, 3-5 and 5-7 minutes post exposure of skin to mosquitoes was recorded. This procedure was repeated twice to obtain three replicates. Olfactometer scores were calculated as described in the procedures for human subjects.

RESULTS AND DISCUSSION

Preliminary studies with the olfactometer: When subject 02 entered the environmental chamber, sat next to the olfactometer, but did not have the forearm under the olfactometer, olfactometer response scores were minimal (0.94) compared to the corresponding scores obtained with the forearm in position (4.37). The olfactometer response score for subject 02 was not diminished by excluding exhaled breath from the environmental chamber. By washing the forearm area a few minutes before placing the test area under the olfactometer, mosquito response to subject 02 was similar to that obtained with the forearm absent. These results indicated that the mosquitoes are responding primarily to emanations from the forearm test area and not from extraneous sources.

Screening of 30 subjects for attractancy: We have identified human subjects from a group of 30 males and females whose forearms were consistently least attractive to Aedes aegypti mosquitoes contained in an olfactometer (Table 1). We also identified subjects who were consistently most attractive to mosquitoes (Table 1). All of the 4 least attractive subjects were female and 10 of the 12 least attractive subjects were female. All of the 5 most attractive subjects were male and 10 of the 12 most attractive subjects were male. Females in general were significantly less attractive to the mosquitoes than the males (ANOVA, $F = 49.33$, $P = 0.0000$). The histograms of olfactometer response for all trials with female subjects is given in Figure 3. The corresponding data for male is given in Figure 4. Olfactometer response did not significantly correlate ($P > 0.05$) with age of male or female subjects (Figures 5 and 6).

Collection and desorption of skin emanations: Volatile emanations from the forearm of subject 02 were captured on Tenax adsorbent and thermally desorbed into the olfactometer over a seven minute period. Between 1 and 3 minutes following the start of desorption, mosquitoes were attracted to the tip of the Tenax trap (Table 2). During the remainder of the desorption period, mosquitoes which entered the lower chamber of the olfactometer (proximal to the desorbing trap) ceased probing towards the tenax trap and began random flight; mosquitoes in the upper chamber of the olfactometer (distal to the desorbing trap) ceased their search for the entry door to the lower chamber and settled on the walls of the distal chamber. Desorption of a blank tenax trap to the same mosquitoes resulted in no observable behavioral response of any kind (Table 2). These results suggest that an attractant chemical or chemicals derived from the forearm were desorbed during the 1-3 minute period. A natural repellent or masker of attraction could have been desorbed during the remainder of the desorption period, although this experiment does not provide unambiguous proof of such an occurrence.

Gas chromatography/mass spectrometry of skin emanations: In a pilot experiment, we collected volatile emanations from the forearm of a male volunteer by passing a stream of high purity nitrogen over the arm and collecting the nitrogen in a 6 liter Summa canister, a fused silica lined container used for field collection of air samples. This sample was transported to the Varian Associates application laboratory (Walnut Creek, CA) for GC/MS analysis on a bench top instrument (Varian Saturn 3). A 25 ml portion of the sample was introduced into the 60 meter DB-1 capillary column of the GC. The chromatogram is given in Figure 7. Total ion current (peak intensity) on the Y axis is plotted vs time and scan number on the X axis . The broad peak starting shortly after scan number 400 is due to water and the spikes between 400 and 800 are mostly hydrocarbons which probably accumulated on the subject's skin during commute to the laboratory. The remaining chromatogram demonstrated that the GC conditions could resolve the sample into distinct peaks and that the instrument (ion-trap GC/MS) had adequate sensitivity, using only 25 ml of the 6000 ml sample from a 5 minute collection. Full scale Y axis in Figure 7 is approximately 10 parts per billion. For demonstration purposes, the mass spectrum of one of the more intense peaks, scan number 1698, is given in the upper graph of Figure 8, along with the mass spectra of possible structures (menthol or menthyl acetate) from the NIST library. Structural assignment would have to be verified with injection of an authentic standard into this instrument. The MS/MS and CI (collision induced ionization) capabilities of this instrument can also aid in structural elucidation. In summary, the pilot experiment demonstrated the feasibility of this analytical approach.

Attractancy of excised pig skin: For many years, we have used pig skin as a model for human skin in permeability studies. It has many similarities to human skin, both anatomical and biochemical[6], and pig skin lipids are very similar to those of humans. When excised pig skin was first presented to Aedes aegypti mosquitoes in the olfactometer, the skin samples were as attractive to the mosquitoes as the average male subject. In fact, a few fugitive mosquitoes attempted to obtain blood meals from the preparation! This was observed for both fresh and frozen skin mounted on diffusion cells containing tissue culture media at 37°C. Under these conditions we have previously shown that freshly excised pig skin maintains its viability for 48 hours, based on the ability of the skin to graft to athymic nude mice at the same success rate as freshly excised pig skin. Biochemical measurements (excretion of lactic acid and RNA metabolites into the tissue culture media) have recently shown that the skin preparation continues to carry out normal biochemical functions over at least a 24 hour period on the diffusion cell[7]. However, the pig skin preparation almost completely lost its attraction to Aedes aegypti over a two hour period on the diffusion cell (Table 3), while the forearm of a volunteer remained attractive. These results suggest the evaporative loss of an attractant that can't be resupplied by the skin preparation; alternatively, the preparation may be emanating a repellent or masker of attraction that is initiated by removal from the donor. In either case, this preparation can serve two useful functions. First, fresh preparations can be used as a model for attraction of mosquitoes to the human forearm. We can therefore employ it to bioassay human forearm emanations for attractant or repellent activity. Second, a differential chemical analysis of emanations during the interval between maximum and zero attraction can help identify compounds that may be similar to human attractants or repellents.

CONCLUSIONS

Among a group of 30 volunteers, certain individuals were found to be consistently least attractive or most attractive to mosquitoes, with males being more attractive than females. It is our hypothesis that this differential attractiveness is due to differences in skin emanations. Chemical and biological characterization of skin emanations from these extreme subjects will test this hypothesis. By knowing the characteristics of natural attractants and/or repellents, we will be able to develop improved protection for both soldiers and civilians.

BIBLIOGRAPHY

1. Bowen, M.F. The sensory physiology of host-seeking behavior in mosquitoes. Annu. Rev. Entomol. 36: 139-158, 1991.

2. Skinner, W.A., H.C. Tong, H. Johnson, R.M. Parkhurst, D. Thomas, T.S. Spencer, W.A. Akers, D. Skidmore and H. Maibach. Influence of human skin surface lipids on protection time of topical mosquito repellents. J. Pharm. Sci., 66: 1764-1766, 1977.

3. Feinsod, F.M., and A. Spielman. An olfactometer for measuring host-seeking behavior of female Aedes aegypti (Diptera: Culicidae). J. Med. Entomol. 15: 282-285, 1979.

4. Compendium Method TO-14. The determination of volatile organic compounds (VOCs) in ambient air using summa passivated canister sampling and gas chromatography analysis, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, May 1988.

5. Hawkins, G.S., and W.G. Reifenrath. The influence of skin source, penetration cell fluid and partition coefficient on in vitro skin penetration. J. Pharm. Sci. 75: 378-381, 1986.

6. Reifenrath, W.G., E.M. Chellquist, E.A. Shipwash, W.W. Jederberg and G.G. Krueger. Percutaneous penetration in the hairless dog, weanling pig and grafted athymic nude mouse. Evaluation of models for predicting skin penetration in man. Brit. J. Dermatol. Suppl. 27: 123-135, 1984.

7. Kammen, H.O., and W.G. Reifenrath, unpublished data, 1996.

8. Brown A.W.A., H.P. Roessler, E.Y. Lipsitz and A.G. Carmichael. Factors in the attractiveness of bodies for mosquitoes. The Canadian Entomologist 96 :102-103, 1964.

9. Rudolphs, W. Chemotropism of mosquitoes. New Jersey Agricultural Experiment Station Bulletin No. 367, 1922.

10. Acree, F, R.B. Turner, H.K. Gouck, M. Beroza and N. Smith. L-Lactic Acid: A mosquito attractant isolated from humans. Science, 161:3846-7, 1968.

11. Van Thiel, P.H. and J.J. Laarman. What are the reactions by which the female Anopheles find its blood supplies? Acta Leidensia 24: 180-187, 1954.

12. Snow, W.F. The effect of a reduction in expired carbon dioxide on the attractiveness of human subjects to mosquitoes. Bull. Ent. Res. 69: 43-48, 1970.

13. Rahm, U. Zum problem der attraktion von stechmucken durch den menschen. Acta Trop. 13:319-344, 1956.

14. Brouwer, R. The attraction of carbon dioxide excreted by the skin of the arm of malaria mosquitoes. Trop Geogr. Med. 12: 62-66, 1960.

15. Khan, A.A., H.I Maibach, W.G. Strauss, and W.R. Fenley. Quantitation of effect of several stimuli on the approach of Aedes aegypti. J. Econ. Entomology 59:690-694, 1966.

16. Carlson, D.A., C.E. Schreck, and R.J. Brenner. Carbon dioxide released from human skin: effect of temperature and insect repellents. Journal of Medical Entomology 29:165-170, 1992.

17. Brouwer, R. Acad. Proefschr. Leiden, 110p, 1958.

18. Schreck, C.E. and J. James. Broth culture of bacteria that attract female mosquitoes. Mosquito News 28: 33-38, 1968.

19. Thompson, R.P. and A.W.A. Brown. The attractiveness of human sweat to mosquitoes and the role of carbon dioxide. Mosquito News 15: 80-84, 1955.

20. Gilbert, I.H. G.K. Gouck and N. Smith. Attractiveness of men and women to Aedes aegypti and relative protection time obtained with DEET. The Florida Entomologist 49: 53-66, 1966.

21. Roessler, P. The attractiveness of steroids and amino acids to female Aedes aegypti. Proceedings of the Fiftieth Annual Meeting, New Jersey Mosquito Extermination Association and Nineteenth Annual Meeting, American Mosquito Control Association, Atlantic City, March 1963, pp 250-255.

22. Price, G.D., N. Smith and D.A. Carlson. The attraction of female mosquitoes (Anopheles quadrimaculatus SAY) to stored human emanation in conjunction with adjusted levels of relative humidity, temperature, and carbon dioxide. J. Chemical Ecology 5: 383-395, 1979.

23. Brown, A.W.A. and A.G. Carmichael. Lysine as a mosquito attractant. Nature 169: 508-509, 1961.

24. Hier, S.W. T. Cornbleet and O. Bergeim. J. Biol. Chem. 166: 327, 1946.

25. Lipsitz, E.Y. and A.W.A. Brown. Studies on the responses of the female Aedes mosquito: IX The mode of attractiveness of lysine and other amino acids. Bull. Entomo. Res. 54 675-687, 1964.

26. Strauss, WG. H.I. Maibach and A.A. Kahn. Drugs and disease as mosquito repellents in man. Am. J. Trop Med. Hyg. 17: 461-464, 1968.

27. Kline, D.L. D.A. Dame and M.V. Meisch. Evaluation of 1-octen-3-ol and carbon dioxide as attractants for mosquitoes associated with irrigated rice fields in Arkansas. J. Am. Mosq. Control Assoc. 7: 165-9, 1991.

28. Maibach, H.I. A.A. Khan, W.G. Strauss and W.A. Skinner. Human skin in relationship to mosquito attraction and repulsion. Connecticut Medicine, 33: 23-28, 1969.

29. Schreck, C.E., N. Smith, D.A. Carlson, G.D. Price, D. Haile and D.R. Godwin. A material isolated from human hands that attracts female mosquitoes. Journal of Chemical Ecology, 8: 429-438, 1981.

30. Skinner, W.A. H.C. Tong, H.I. Maibach and D. Skidmore. Human skin-surface lipid fatty acids - mosquito repellents. Experientia 26: 728-730, 1970.

31. Skinner, W.A., H.C Tong, H. Johnson, R.M. Parkhurst, D. Thomas, T. Spencer, W. Akers, D. Skidmore and H. Maibach. Influence of human skin surface lipids on protection time of topical mosquito repellent. J. Pharm. Sci., 66: 1764-1766, 1977.

32. Takken, W. The role of olfaction in host-seeking of mosquitoes: a review. Insect Sci. Applic. 12: 287-295, 1991.

33. Bowen, M.F. The sensory physiology of host-seeking behavior in mosquitoes. Annu. Rev. Entomol. 36: 139-158, 1991.

Table I. Olfactometer response to *Aedes aegypti* mosquitoes.

Subject No.	Olfactometer Response[a]	No. of Replicates
30 (Female)[b]	1.73 ± 0.67	3[c]
24 (Female)[b]	2.13 ± 1.13	9
15 (Female)[b]	2.65 ± 0.53	8
29 (Female)[b]	2.79 ± 1.44	9
18 (Female)	3.01 ± 1.19	9
26 (Male)	3.06 ± 0.97	9
16 (Female)	3.26 ± 1.10	9
1 (Female)	3.34 ± 1.35	18
3 (Male)	3.47 ± 1.52	10
27 (Female)	3.56 ± 1.39	9
11 (Female)	3.60 ± 1.19	9
28 (Female)	3.65 ± 0.53	6
25 (Male)	3.67 ± 1.49	6
23 (Male)	3.82 ± 1.05	9
12 (Female)	4.08 ± 1.18	9
10 (Female)	4.22 ± 1.63	3
22 (Male)	4.25 ± 0.92	9
17 (Male)	4.33 ± 0.94	9
6 (Female)	4.39 ± 1.51	9
13 (Male)	4.44 ± 1.36	9
5 (Male)	4.45 ± 0.62	9
20 (Male)	4.74 ± 0.68	9
4 (Female)	4.92 ± 0.88	9
19 (Male)	4.93 ± 0.99	3
21 (Male)	5.03 ± 0.84	6
14 (Male)[b]	5.06 ± 1.11	9
7 (Male)[b]	5.20 ± 1.11	9
9 (Male)[b]	5.21 ± 0.85	9
2 (Male)[b]	5.31 ± 0.73	11
8 (Male)[b]	5.32 ± 0.76	9

[a]Olfactometer response (mean $\pm$ S.D.) was calculated as the area under the curve of fractional mosquito response versus time profile. A hypothetical test subject completely unattractive to mosquitoes would have a score of zero. A maximally attractive subject would have a score of almost seven.

[b]Olfactometer response scores were analyzed by ANOVA and the Student-Newman-Keuls Multiple Range Test, which identified subjects Nos. 15, 24, 29, and 30 as least attractive to mosquitoes and subjects Nos. 2, 7, 8, 9, and 14 as most attractive to mosquitoes. Each of the four least attractive female subjects were significantly different from all of the five most attractive male subjects (Tukey's test, $P < 0.05$).

[c]Subject 30 was retested on a separate occasion with even lower olfactometer scores; however, the mosquitoes were exposed to low temperatures from an equipment malfunction and the results are not included.

Table II. Cumulative response of Aedes aegypti mosquitoes to thermal desorption of skin emanations into an olfactometer.

Condition	T= 0 min Percent Response	T= 1 min Percent Response	T= 3 min Percent Response	T= 5 min Percent Response	T= 7 min Percent Response
Blank Trap Desorption	0%	0%	0%	0%	0%
Forearm Emanation Desorption	0%	8%	23%	31%	31%

Table III. Olfactometer response of *Aedes aegypti* to excised pig skin as a function of time after placing skin on a diffusion cell

Trial	Time After Preparation	Olfactometer Score
1	T = 0 h	3.40
2	T = 1 h	1.95
3	T = 2 h	0.23

Figure Legends

Figure 1. Modified Feinsod-Spielman olfactometer

Figure 2. Skin penetration cell for excised pig skin preparation

Figure 3. Histogram of olfactometer scores (attractancy) of female test subjects. A higher number designates greater attractancy.

Figure 4. Histogram of olfactometer scores (attractancy) of male test subjects. A higher number designates greater attractancy.

Figure 5. Plot of female test subject olfactometer score (attractancy) vs age.

Figure 6. Plot of male test subject olfactometer score (attractancy) vs age.

Figure 7. Total ion chromatogram of forearm emanations from subject 02.

Figure 8. Mass spectrum of peak at scan number 1698 (upper spectrum) and possible matches from the NIST library (lower 3 spectra). See Figure 7 for corresponding chromatogram.

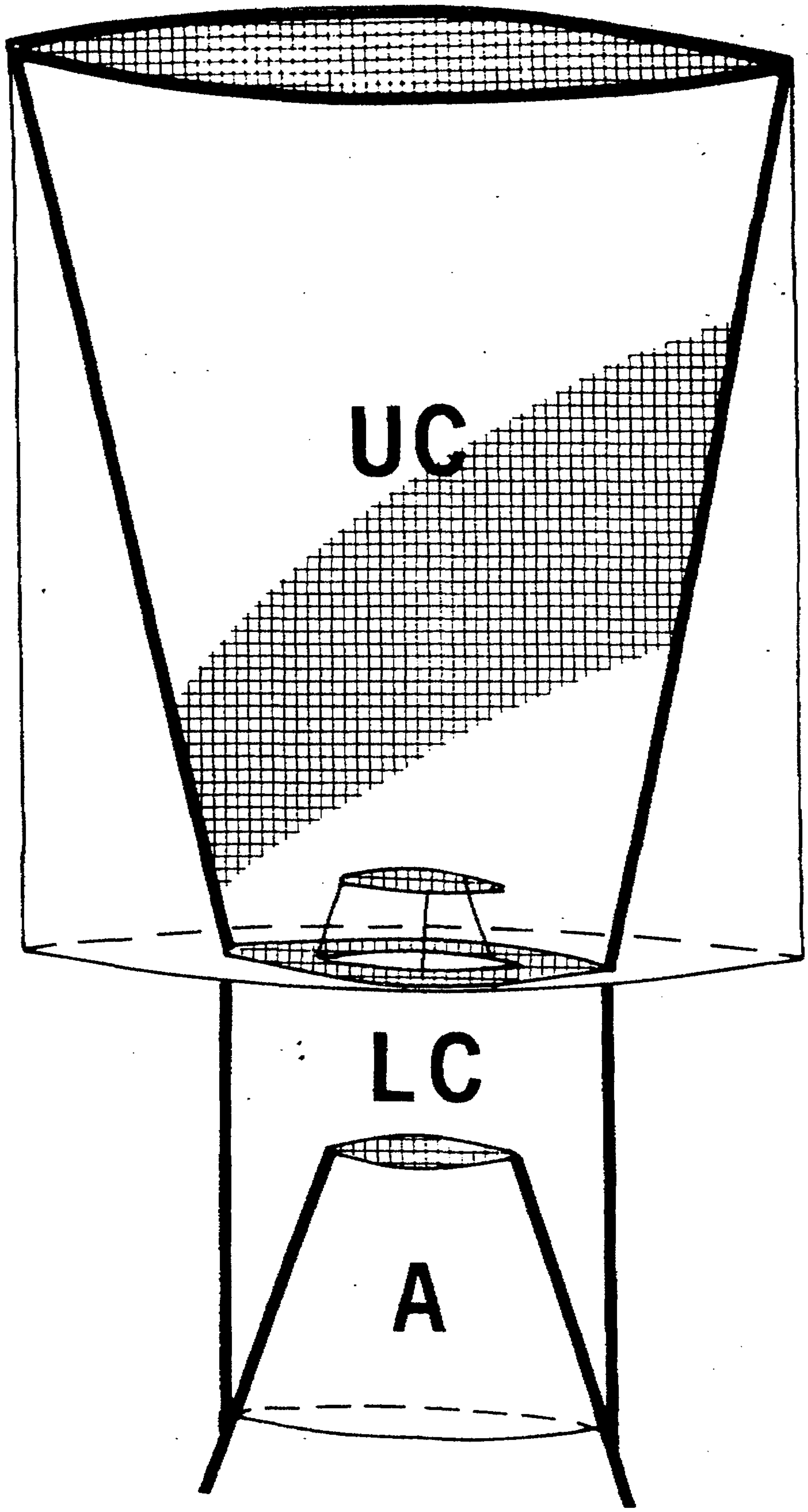

Figure 1. Modified Feinsod-Spielman olfactometer.

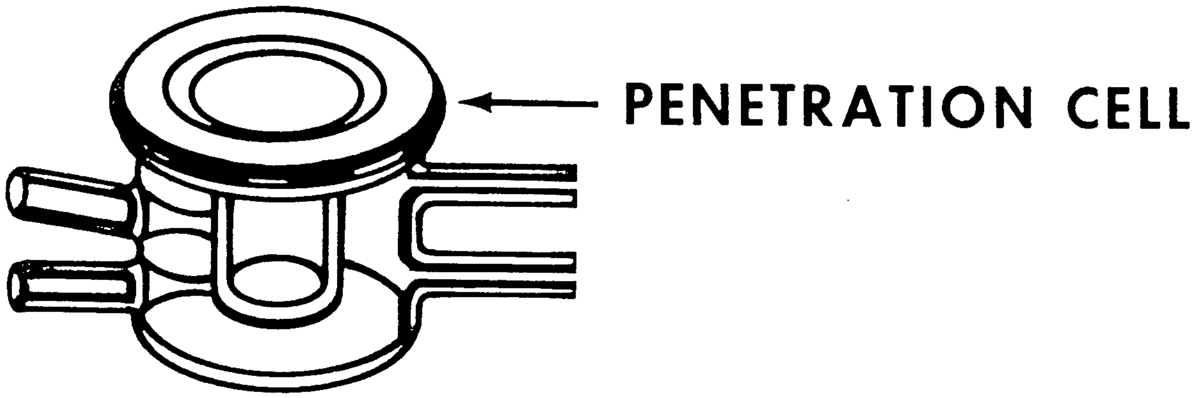

Figure 2. Skin penetration cell for excised pig skin preparation.

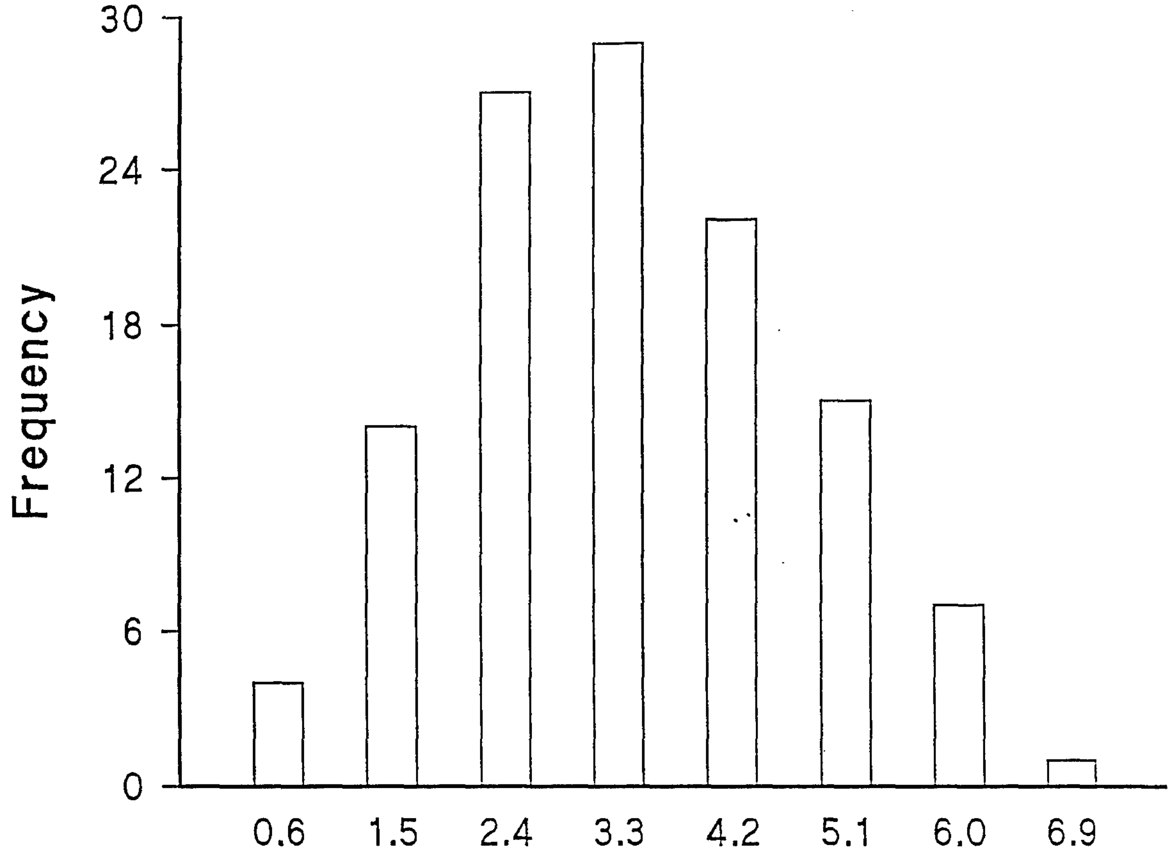

Figure 3

Female Olfactometer Score
N = 119 Total Measurements

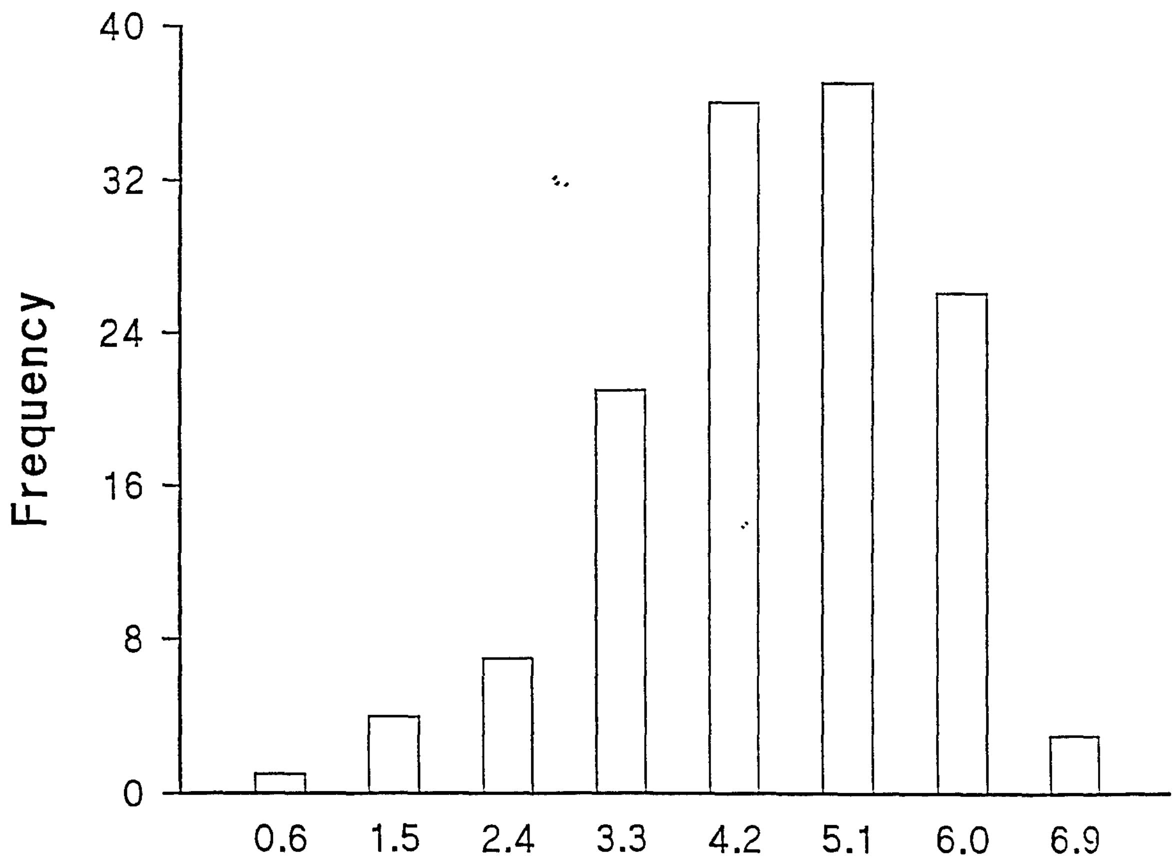

Figure 4

Male Olfactometer Score
N = 135 Total Measurements

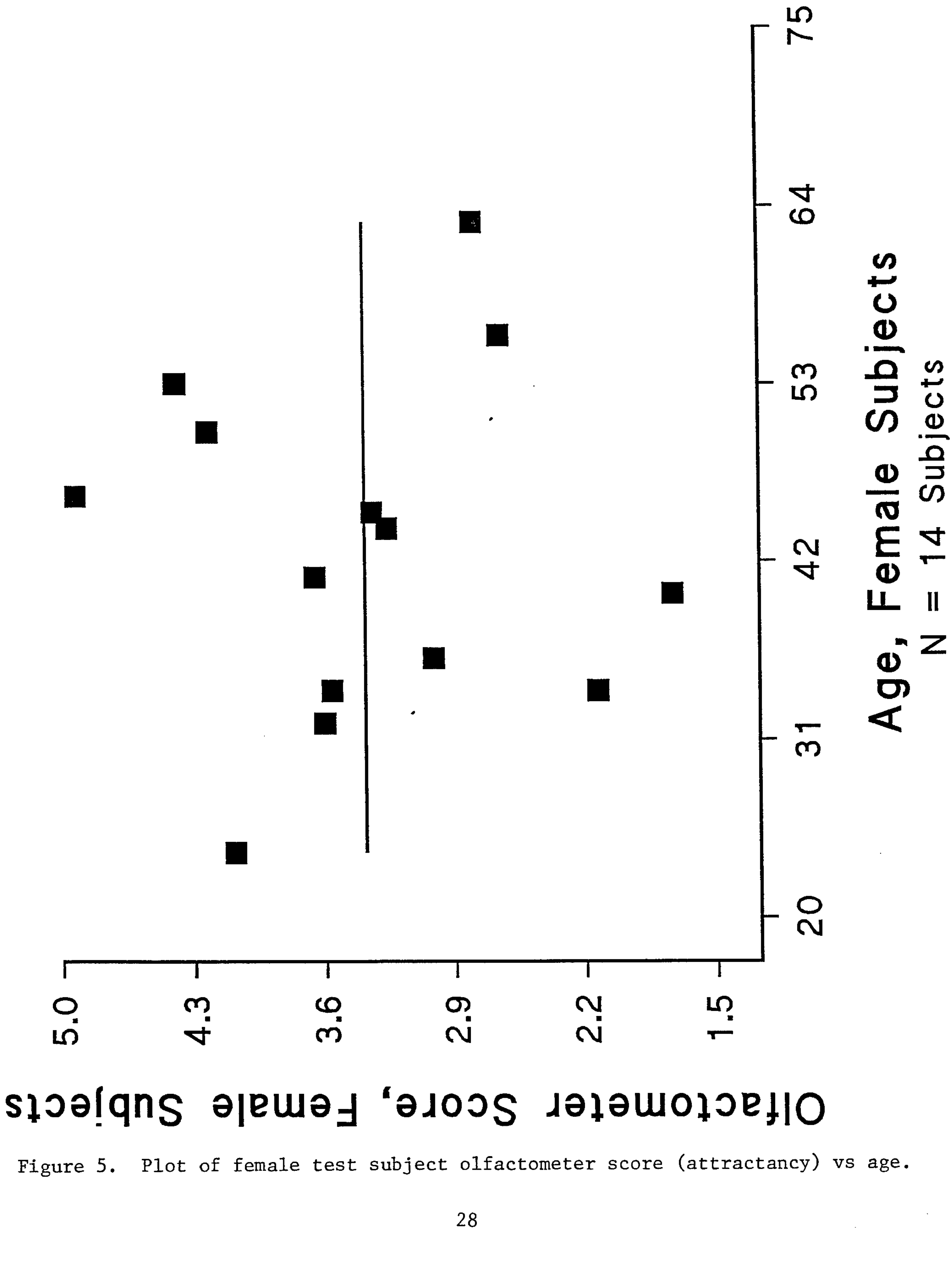

Figure 5. Plot of female test subject olfactometer score (attractancy) vs age.

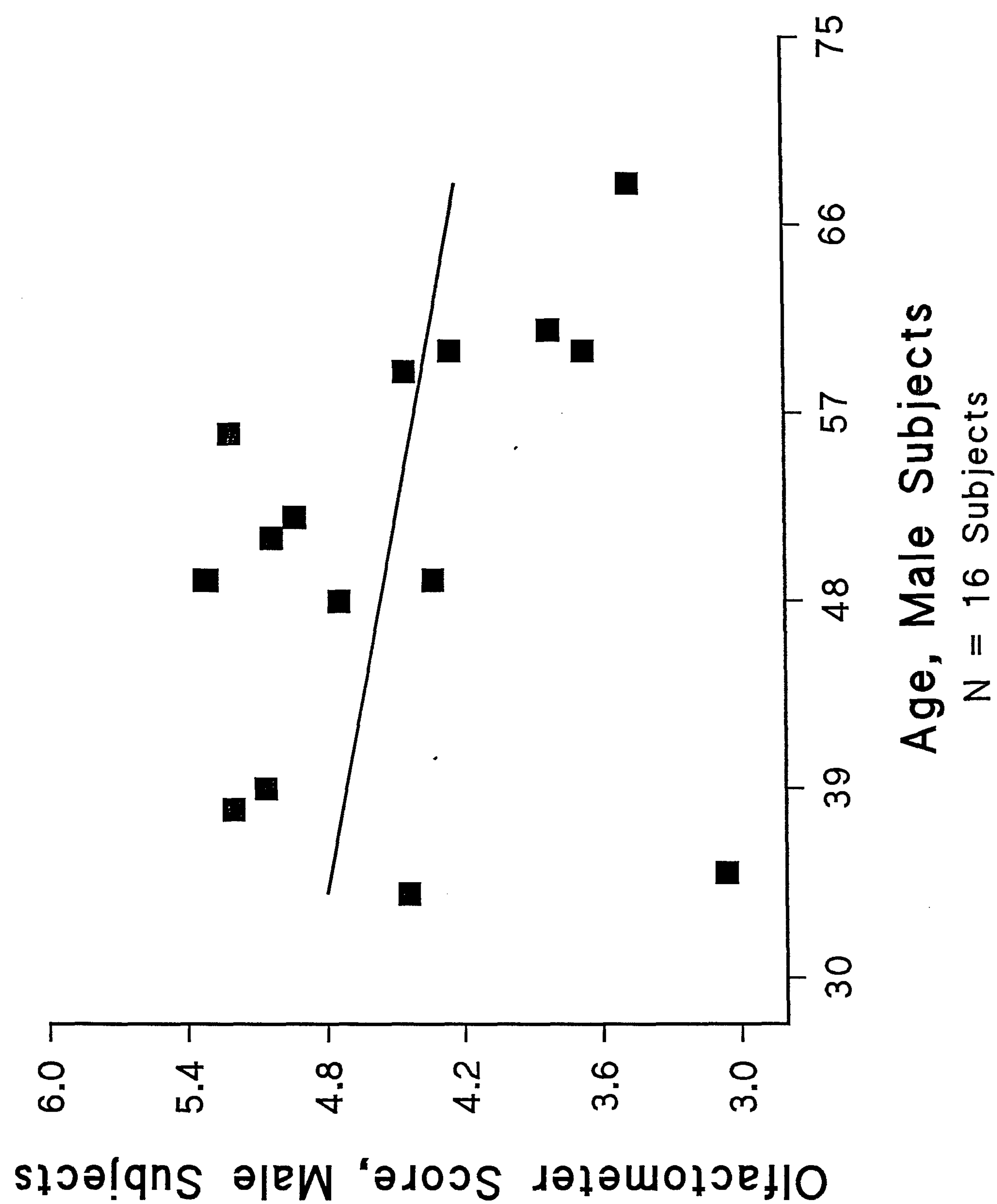

Figure 6. Plot of male test subject olfactometer score (attractancy) vs age

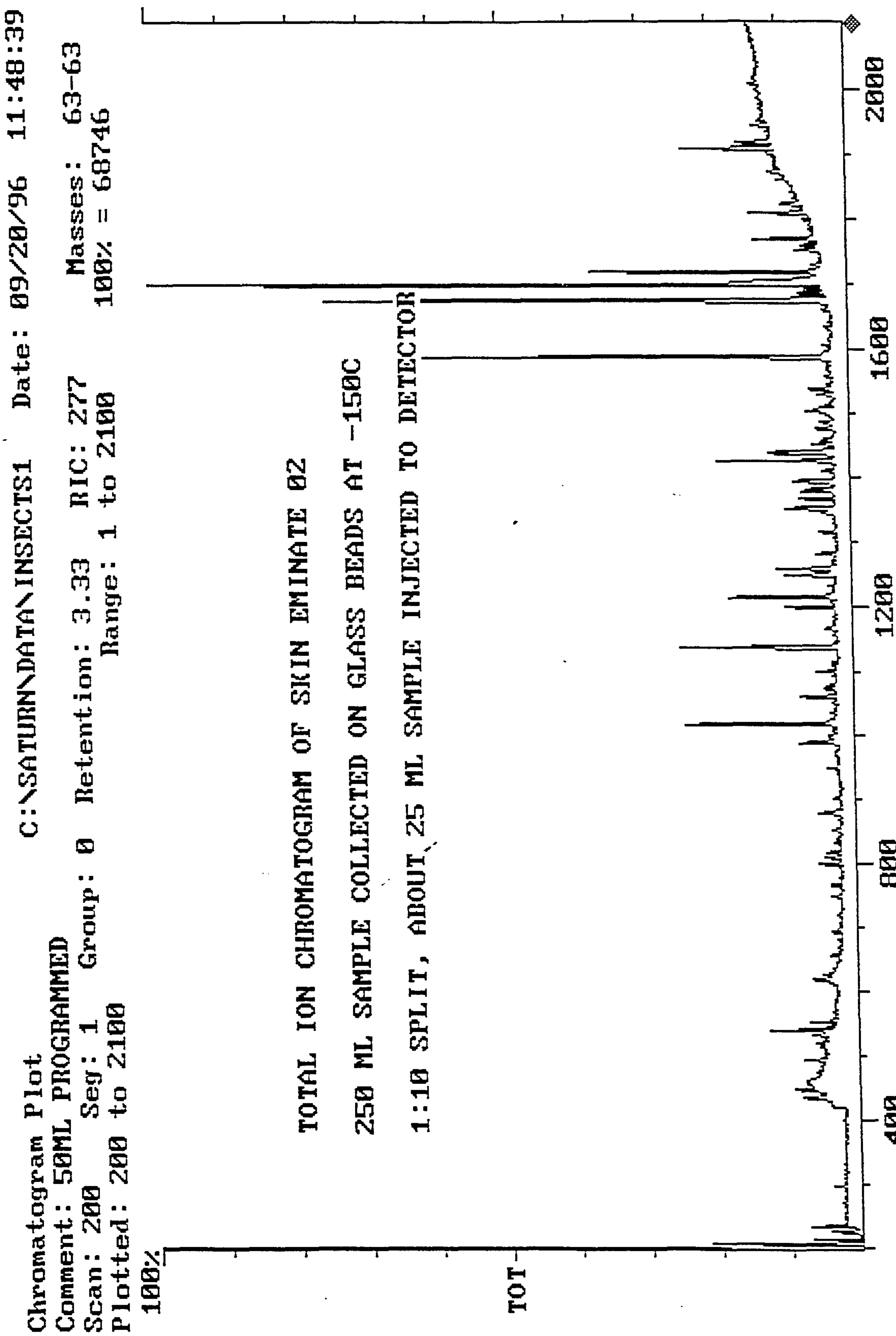

Fig 7. Total ion chromatogram of forearm emanations from subject 02.

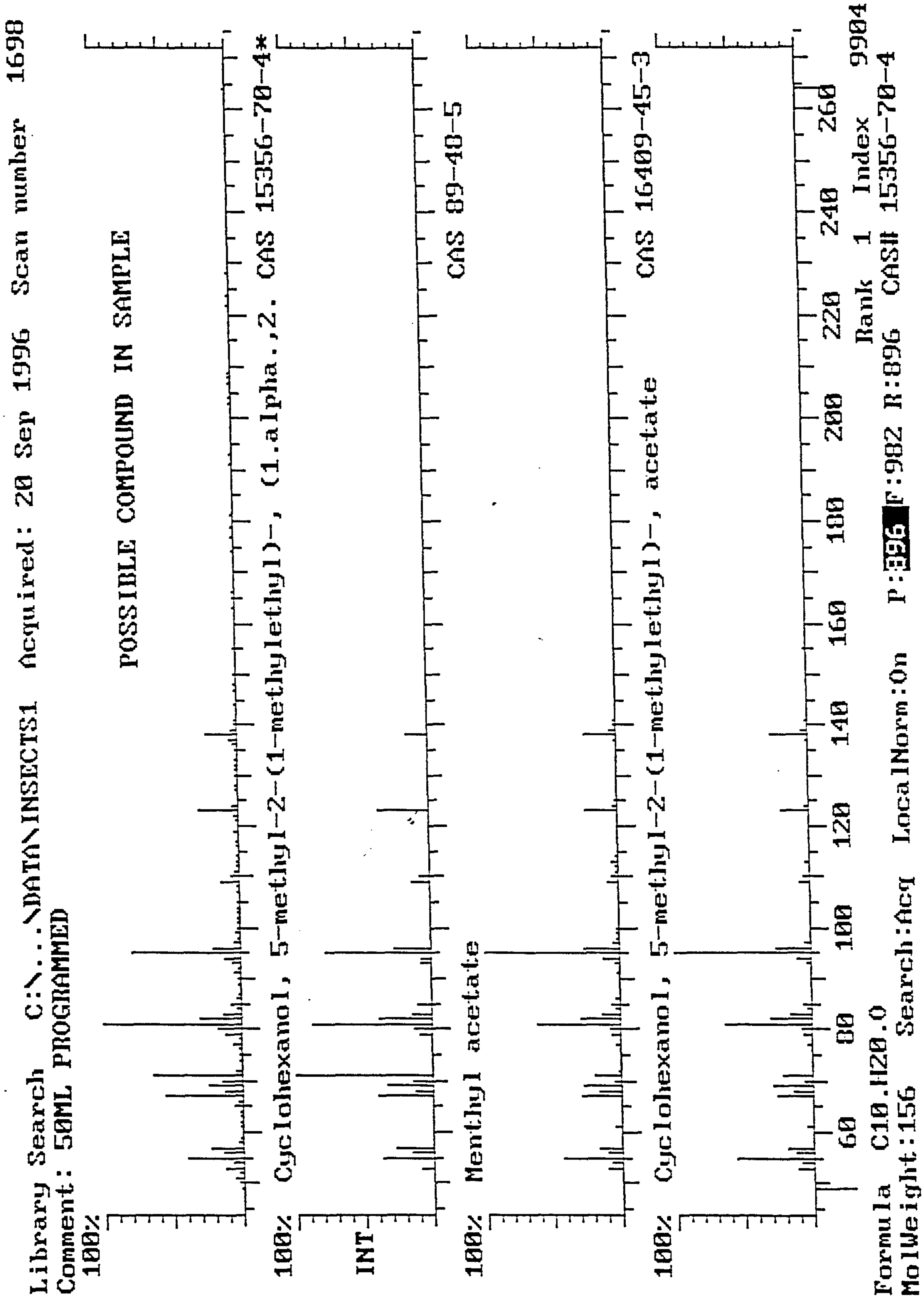

Fig 8. Mass spectrum of peak at scan number 1698 (upper spectrum) and possible matches from the NIST library (lower 3 spectra)

APPENDIX A. SURVEY OF LITERATURE

HUMAN SKIN ATTRACTANTS AND REPELLENTS FOR MOSQUITOES

Natural Attractants/Repellents of Human Skin to Mosquitoes Brown[8] lists a number of factors involved in the attraction of mosquito to man (in order of importance): moisture, convective heat, carbon dioxide, movement, contour or increase in black-white interfaces, and reflectivity. The influence of carbon dioxide as a mosquito "activator" has long been recognized[9]. However, Acree[10] and coworkers have shown that carbon dioxide does not attract mosquitoes in purified air alone. Thiel and Laarman found that air swept over the arm was attractive even though carbon dioxide and moisture had been removed; they concluded the presence of other attractants or odors was responsible for the attraction[11]. Snow[12] studied mosquito attraction to normal subjects and to subjects wearing a breathing apparatus to remove most of the exhaled carbon dioxide. Fewer mosquitoes were attracted to the subjects with reduced carbon dioxide output. However, when the mosquitoes were in close range of the host, the experimental treatment had no effect on the proportion of mosquitoes attempting to feed. Snow concludes from this study that carbon dioxide, originating from the lung, may be more important as a long range attractant. Reports by Rahm[13] and Brouwer[14] showed that carbon dioxide output from the skin was insignificant in stimulating mosquitoes. In contrast to these findings, Khan et al[15] concluded that heat and carbon dioxide are important for the approach of mosquitoes to the host at close proximity, and that odor was more important at greater distance. Carlson et al[16] measured the amount of carbon dioxide given off by the hand at 1.0-1.8 ml/h under laboratory conditions. The authors concluded that this amount of carbon dioxide is negligible compared to ambient levels and was unlikely to be attractive to mosquitoes by itself.

In 1958, Brouwer[17] reported consistent differences in attraction of Anopheles stephensi to humans that were independent of moisture, warmth and carbon dioxide. He concluded that the differences were due to sweat or body odor. Schreck[18] reported that a polyethylene glove, worn for 1 hour, remained attractant to mosquitoes over a 3 hour period after removal from the hand. Thompson and Brown[19] demonstrated the attractiveness of sweat was decreased by the release of volatile acids.

Gilbert[20] et al. studied 50 men and 50 women to determine their attractiveness to Aedes aegypti mosquitoes. The 50 women subjects were, on average, less attractive than the 50 men. However, there was considerable overlap in the ranges of attraction, and many of the women were more attractive than some of the men. However, only two of the most attractive 10 subjects were women, and all of the least attractive 10 were women. A possible relationship between attraction and differences in skin lipid composition was not investigated. Roessler[21] hypothesized that changes in the attractiveness of females with the menstrual cycle were caused by changes in estrogen evaporation from the skin.

In a 1968 report, Acree et al.[3] found a correlation between the attractiveness of individuals to mosquitoes and the quantity of lactic acid present in acetone washings of hands. Attractive material was first obtained by condensation of a nitrogen stream above the skin. However, the amount of material obtained was too small for analytical methods available at that time. These workers noted that the attractancy of lactic acid was not evident without the presence of carbon dioxide.

Price[22] et al studied the attraction of mosquitoes to human emanations in a dual port olfactometer. Mosquitoes (female Anopheles quadrimaculatus SAY) were preferentially attracted to the "emanation" air, even though excess carbon dioxide or water had been added to control air without emanations.

In 1961, Brown and Carmichael[23] reported that lysine free base was a mosquito attractant. Lysine was known to be present in human sweat[24]. Although other amino acids had mosquito attractant properties, they were considerably less attractant than lysine. The attractiveness of lysine was later found to be proportional to the presence of carbon dioxide[25]

Strauss et al.[26] , surveyed hospitalized patients with various diseases and taking various medications for their attractiveness to mosquitoes by a mosquito probing technique. No drug, vitamin, or disease was associated with unattractiveness, with the possible exception of untreated myxedema.

In addition to the compounds mentioned above, USDA investigators have studied 1-octen-3-ol as a mosquito attractant[27]. Israeli investigators found that although sheep were attractive to Culex pipiens L. and Aedes caspius (Pallas), few Culex pipiens and no Aedes caspius engorged. The investigators suggested that sheep may possess, in addition to the mechanical protection afforded by wool, a close-acting repellent that deters the mosquitoes from biting. The repellent was not identified.

Maibach and coworkers[28] report the observation that the attractancy of human sweat increased significantly when lipids were removed. Schreck and coworkers[29] isolated a material from glass beads previously handled by humans. This residue was found to be attractant to female Aedes aegypti and Anopheles quadrimaculatus Say mosquitoes. This residue was characterized as volatile, and stable on refrigerated storage for up to 60 days. The residue was not purified or chemically analyzed. Skinner et al[30] obtained human skin-surface lipids from ether washings of elbows from a number of volunteers. This mixture was found to be repellent to Aedes aegypti mosquitoes. Vacuum distillation, gas chromatography and thin layer chromatography were used to isolate components from the mixture. The hydrocarbon fraction of the lipids contained only weakly repellent unsaturated hydrocarbons, with the major repellent activity present in the more polar fractions. The unsaturated fatty acids were found to be more repellent than saturated fatty acids. In a later report (1977), Skinner et al[31] analyzed acetone extracted lipids from skin using gas chromatography-mass spectroscopy. Multiple regression analysis was used to relate attractancy and repellent protection time to the amounts of saturated and unsaturated fatty acids. Dry protection time or duration of protection of the insect repellent N,N-diethyl-3-benzamide (DEET) correlated positively with saturated fatty acids C-11, C-13, C-15 and C-18 and unsaturated fatty acids C-14, C-15, C-16 and C-17; dry protection time correlated negatively with saturated C-7, C-12 and C-16 fatty acids. The fatty acids may affect the protection time of DEET by a physical mechanism; that is, they may alter the evaporation and penetration of DEET through their film forming activity. Indeed, repellent protection time of DEET correlated positively with the total weight of lipid found on the skin. Attractancy, as measured by the average number of Aedes aegypti mosquitoes probing the test site of the volunteer in one minute, was found to correlate positively with C-15 unsaturated fatty acid and C-14 saturated fatty acid; attractancy was found to correlate negatively with the more volatile C-11 saturated fatty acid. The authors indicated that the precise identification of fatty acid components affecting attractiveness would require further study.

Studies of human skin emanations associated with mosquito attraction/repulsion have recently been reviewed[32].

In summary, there is ample evidence that human skin emanates both attractant and repellent compounds for mosquitoes. No single compound is likely responsible for mosquito attraction; the same can be said for mosquito repulsion. The interaction of these compounds is probably of importance in the overall response of the mosquito.

Skin emanations have been poorly characterized[33]. While a few studies have chemically characterized skin surface extracts, it is important to characterize what the skin is actually emanating; that is, what chemicals the mosquito actually senses during its host seeking behavior, rather than residues on the skin. Some studies have utilized a solvent evaporation step in the process of characterizing skin residues; important volatile components could have been lost in the process. Finally, gas chromatographic-mass spectrometric techniques have been greatly advanced since the earlier studies were conducted, both in terms of sensitivity and software for analysis of mixtures.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Phyllis Rinehart

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

ADB218773
ADB223531
ADB230017
ADB223528
ADB231930
ADB226038
ADB224296
ADB228898
ADB216077
ADB218568
ADB216713
ADB216627
ADB215717
ADB218709
ADB216942
ADB216071
ADB215736
ADB216715
ADB215485
ADB215487
ADB220304
ADB215719
ADB216072
ADB222892
ADB215914
ADB222994
ADB216066
ADB217309
ADB216726
ADB216947
ADB227451
ADB229334
ADB228982
ADB227216
ADB224877
ADB224876
ADB227768
ADB228161
ADB229442
ADB230946
ADB230047
ADB225895
ADB229467
ADB224342
ADB230950
ADB227185
ADB231856

ADB229914
ADB229497
ADB230947
ADB282209
ADB270846
ADB282266
ADB262442
ADB256670